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Genome-wide Association Study Identifies a Novel Maternal Gene × Stress Interaction Associated with Spontaneous Preterm Birth

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Author Contribution

All authors meet the journal's authorship requirements and approved the final manuscript. X.W. is the principal investigator of the Boston Birth Cohort (the parent study) and obtained the study funding to support the study cohort. The subject recruitment and data collection were overseen by XW and conducted by a team of investigators including X.H., Y.J., B.Z., et al. X.H. and X.W. took primary responsibility for the study design, have full access to all of the data in the study and are responsible for the accuracy of the data analyses developed the concept for this analysis. X.H. and P.J.S. drafted the manuscript. X.H., B.Y.Z. and Y.J. conducted the data analysis, with guidance from H.J.. C.P., C.S. and B.Z. supervised the field data collection. All authors contributed to the analysis plan, data interpretation, and critically reviewed and edited the manuscript.

Patient consent

Each participant provided written informed consent prior to participation. The study protocol was approved by the Institutional Review Boards of Boston University Medical Center, and of the Johns Hopkins Bloomberg School of Public Health.

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Abstract

Background—Maternal stress is potentially a modifiable risk factor for spontaneous preterm birth (sPTB). However, Epidemiologic findings on the maternal stress - sPTB relationship have been inconsistent.

Methods—To investigate whether the maternal stress - sPTB associations may be modified by genetic susceptibility, we performed genome-wide gene×stress interaction analyses in 1,490 African-American women from the Boston Birth cohort who delivered term (n=1,033) or preterm (n=457) infants. Genotyping was performed using Illumina HumanOmni 2.5 array. Replication was performed using data from the NICHD genomic and Proteomic Network (GPN) for PTB Research.

Results—rs35331017, a T-allele insertion/deletion polymorphism in the protein-tyrosine phosphatase receptor Type D *(PTPRD)* gene, was the top hit that interacted significantly with maternal lifetime stress on risk of sPTB ($P_{G\times E}$ =4.7×10^{-8}). We revealed a dose-responsive association between degree of stress and risk of sPTB in mothers carrying the insertion/insertion genotype; but an inverse association was observed in mothers carrying the heterozygous or deletion/deletion genotypes. This interaction was replicated in African-American ($P_{G\times E}$ =0.088) and Caucasian mothers ($P_{G\times E}$ =0.023) from the GPN study.

Conclusion—We demonstrated a significant maternal *PTPRD* × stress interaction on sPTB risk. This finding, if further confirmed, may provide new insight into individual susceptibility to stress-induced sPTB.

Introduction

In the United States, more than 10% of all live births are preterm (defined as < 37 completed weeks of gestation). Preterm birth (PTB) remains a leading cause of infant mortality and morbidity in the US and the globe,(1, 2) and can lead to a cascade of health problems later in life.(3, 4) Of all PTBs, about 70% are spontaneous with either preterm labor (that is, having regular contractions and cervical changes at < 37 weeks of gestation) or preterm premature rupture of membranes, and the remaining PTBs are medically indicated that occur largely due to gestational complications. Women of African ancestry are known to bear a disproportionally high rate of PTB than women of other ethnicities.(5) However, risk factors of PTB that may underpin this disparity remain largely unknown.

Maternal perceived stress, in the form of acute, chronic, pregnancy-related and/or other life event-related stressors, is widespread in the general population and even more so among African Americans (AAs).(6) Maternal stress is potentially an important modifiable social

determinant of maternal and child health in the US.(7) Although many epidemiologic studies have attempted to link maternal stress with risk of PTB, the results have been inconclusive.(7–14) Such inconsistencies may be due to multiple factors. Asides from study design or methodological issues, one possibility that contributes to this inconsistency is the effect modification by maternal genetic susceptibility. It is likely that women of different genetic backgrounds vary in their biological vulnerability to stress, leading to differences in stress - PTB associations. Boyce has proposed the “orchid vs dandelion” theory,(15) which also suggests that certain genetic variants can increase a person’s susceptibility to stressors. This plausibility was further supported by previous epidemiological studies that demonstrated a significant impact of the interaction between maternal genes and perceived stress on multiple child health outcomes. (16–18) Although there is an increasing number of genome-wide association studies (GWAS) of PTB (19, 20), few studies have been conducted to systematically investigate maternal gene × stress interactions on PTB, especially on a genome-wide scale.

To fill the aforementioned research gap, in this study, we performed genome-wide interaction analyses to explore common single nucleotide polymorphisms (SNPs) that may interact with maternal perceived stress (lifetime and during pregnancy) to affect PTB risk in African American women. We used a two-stage case-control study design (including the discovery and the replication stages) and focused on spontaneous PTB (sPTB) to reduce phenotypic heterogeneity. We further performed a meta-analysis combining the discovery and replication samples.

Materials and Methods

The study cohort and study population in the discovery stage

The study participants in the discovery stage were enrolled in the Boston Birth Cohort (BBC), an ongoing longitudinal, multi-ethnic, predominantly urban, low income minority birth cohort designed to identify gene-environment interactions associated with prematurity and other adverse birth outcomes.(21) Briefly, since 1998, mother-infant dyads have been recruited 1–3 days post-delivery and interviewed using a standard questionnaire at Boston Medical Center. Pregnancies resulting from in-vitro fertilization, multiple gestations, and/or pregnancies with fetal chromosomal abnormalities or major birth defects were excluded. After giving written informed consent, each enrolled mother was interviewed using a standard questionnaire to collect data on demographic variables, lifestyle and dietary intake. A maternal blood sample was obtained within 24–72 hours after delivery and an umbilical cord blood sample was obtained at delivery. The study protocol was approved by the Institutional Review Boards of Boston University Medical Center, and of the Johns Hopkins Bloomberg School of Public Health.

As we reported previously(22), 698 biologically-unrelated AA mothers of preterm babies (PTB, < 36.8 weeks of gestation) and 1,035 AA mothers of term babies (TB, > 37 weeks of gestation) who are frequency matched with the cases on maternal country of origin (Haitian or non-Haitian), maternal age at delivery (+/− 5 years), parity and year of delivery, were successfully genotyped for the GWAS of PTB in the BBC. After removing 237 mothers with medically-indicated PTB and 6 mothers with missing data for maternal perceived stress, this

study included 457 mothers of sPTB (cases) and 1033 mothers of TBs (controls) as the discovery sample.

Phenotype definition and covariates

Gestational age was assessed by early (<20 weeks) prenatal ultrasound and/or the first day of the last menstrual period. Spontaneous PTB (sPTB) was defined as a birth occurring secondary to documented active preterm labor (uterine contractions with cervical effacement and dilation at <37 weeks) or premature rupture of membranes at <37 weeks without uterine contractions or both. Early sPTB was defined as a birth occurring <33 weeks of gestation, and late sPTB as a birth occurring from 33 to $36^{6/7}$ weeks.

Maternal perceived lifetime stress and stress during pregnancy were self-reported by mothers using a standard questionnaire interview, with the following two questions: (1) “how would you characterize the amount of stress in your life in general?” and (2) “How would you characterize the amount of stress in your life during this pregnancy?”. The response options for both questions included: 0 = not stressful (or low), 1 = average, or 2 = very stressful (or high). Other maternal characteristic variables were also collected through a questionnaire interview,(23) including: smoking during pregnancy, which was classified as “never smoker” (did not smoke cigarettes throughout the index pregnancy), “former smoker” (only smoked in the 3 months before pregnancy or during the first trimester), or “continuous smoker” (smoked continuously from pre-pregnancy to delivery); and, social support from the baby’s father, which was classified as “none”, “a little”, “a good amount”, or “an excellent amount”. Pre-pregnancy body mass index (BMI) was calculated as self-reported pre-pregnancy weight (kg) divided by height squared (m^2).

Genome-wide genotyping and genetic ancestry estimation

DNA samples from the proposed mothers were quantified and then genotyped by the Center for Inherited Disease Research using the Illumina HumanOmni 2.5 array. The raw genome-wide genotyping and phenotypic data from the BBC have been deposited in the NIH dbGaP database (entry # phs000332.V3.P2). Detailed information on genotyping and quality control steps can be found in our previous publication.(22) A total of 2,160,368 SNPs from 1,733 biologically unrelated mothers passed the quality control steps and were available for SNP imputation. Phasing was performed using SHAPEIT(24) and SNP imputation was done using IMPUTE2(25) software, with all individuals in the 1000 Genomes Project (1000GP) as the reference panel.

Genetic ancestry for each subject was then computed by principal component analysis (PCA) using Eigenstrat (26), with all individuals in the 1000GP as the reference. Those mothers (n=9) whose estimated genetic ancestry was inconsistent with self-reported African American ancestry were removed from the subsequent data analyses, as we previously reported. (22) The estimated genetic ancestry, represented by the first three principal components from PCAs, was then included as three covariates in subsequent analyses.

Statistical analyses in the discovery stage

Population characteristics in the PTB case and control groups were compared using t-tests for continuous variables or chi-square tests for categorical variables. The genome-wide genotyped SNP × stress interactions were tested using the conventional 1-degree of freedom (df) interaction test. We added each SNP (under an additive genetic model, all with minor allele frequency [MAF] > 2%), maternal perceived stress (lifetime or during pregnancy, coded as 0=low, 1=average, 2=high, and treated as an ordered discrete variable) and their interaction term into a logistic regression model in PLINK(v1.07) (27), with adjustment of covariates including genotyping batch, maternal genetic ancestry, age at delivery, marital status, parity, social support from the baby's father and newborn sex. The genome-wide suggestive and significance thresholds were set as $P<5.0\times10^{-7}$ and $P<5.0\times10^{-8}$, respectively. Manhattan and quantile-quantile (Q-Q) plots were generated using the R package GWASTools (28) to present genome-wide interaction associations. For any genomic loci having significant or suggestive interactions with maternal stress, imputed SNPs nearby (+/− 1Mb), with MAF > 0.02, were further analyzed for their interactions with maternal stress on risk of sPTB using the logistic regression model as described above. The LocusZoom plot was then generated using a published web tool(29) to locate the most significant locus or SNP.

The identified interactions in the discovery sample were validated in the replication samples as describe below. For the validated gene × stress interactions, additional analyses were also performed to test whether the identified interaction varied by different PTB subtypes (i.e., early vs late sPTB) or by newborn sex.

Replication Studies

The replication study was conducted using the epidemiological and GWAS data from the NICHD Genomic and Proteomic Network for Preterm Birth Research (the GPN study, dbGaP entry #phs000714.v1.p1). The GPN study, as reported previously by Zhang et al (30), is to investigate genome-wide associations of sPTB in multi-ethnic populations. Mothers of sPTB (defined as birth at 20 – $33^{6/7}$ weeks) and of TB controls (defined as birth at 39 – $41^{6/7}$ weeks) were matched on race/ethnicity, maternal age, and parity, and genotyping was performed using Affymetrix Genome-wide Human SNP Array 6.0. After data cleaning using similar criteria as in the BBC samples and removing individuals with missing data on maternal stress or on the targeted SNP, the GPN study had 337 AA mothers and 738 Caucasian mothers for replication. Maternal stress was self-reported based on questionnaire interview, with the question about "felt nervous and stressed?". Due to the limited number of AA mothers, we recoded maternal stress into three categories: "low" (if the mothers reported "never" or "almost never" stressed), "average"(if mothers reported "sometimes" or "fairly often")," or "high" (if mothers reported "very often"). The gene × stress interactions were analyzed using the conventional 1-d.f. test based on the logistic regression model, with the adjustment of genetic ancestry, maternal age at enrollment, parity, marital status, and newborn sex. For the imputed SNP *rs35331017*, the best-guessed genotype was applied for interaction tests.

Results

Population characteristics of the discovery sample

After data cleaning steps (see “Materials and Methods”), the discovery sample of this study included 457 AA mothers of sPTB (cases) and 1033 AA mothers delivering at term (controls) from the BBC. Their population characteristics are presented in Table 1. Compared to controls, mothers of sPTB were more likely to have high lifetime stress (16.2% vs 10.2%, P=0.001), to be unmarried (72.9% vs 66.4%, P=0.013) and to smoke during pregnancy (17.1% vs 9.4%, P <0.001); and mothers of sPTB were less likely to receive substantial support from the baby’s father (P=0.001) or from other family members/friends (P =0.003). In comparison, maternal stress during pregnancy was only marginally different between these two groups (23.6% vs 18.1%, P=0.071).

Genome-wide screening for maternal SNP × maternal perceived stress interactions

At the discovery stage, we analyzed genome-wide SNP interactions with maternal lifetime stress and with maternal stress during pregnancy, separately, on risk of sPTB. After adjustment for covariates (see “Materials and Methods”), we found a suggestive genome-wide interaction between *rs11795005* at 9p24.1-p23 and maternal lifetime stress on risk of sPTB ($P_{G\times E}$ =1.4×10^{-7}, Figure 1a). There was no evidence of genomic inflation in our data analyses, as demonstrated by the Q-Q plot (Figure 1b). The identified SNP, *rs11795005*, is located within an intronic region of the protein-tyrosine phosphatase receptor Type D *(PTPRD)* gene. Following SNP imputation of this genomic region, we identified another SNP, *rs35331017,* that demonstrated a genome-wide significant interaction with maternal lifetime stress ($P_{G\times E}$ =4.7×10^{-8}, Fig. 1c) on risk of sPTB. Of note, *rs35331017* and *rs11795005* are in significant linkage disequilibrium (r=0.97). In comparison, we did not identify any genomic significant or suggestive regions interacting with maternal stress during pregnancy on risk of sPTB (Supplementary Fig S1).

Rs35331017 × maternal stress interaction and sensitivity analyses

SNP *rs35331017* is a T-nucleotide insertion (I) / deletion (D) polymorphism with a minor allele (or D allele) frequency of 5% in the BBC. This variant was analyzed under a dominant model (the II genotype versus the ID/DD genotype) in subsequent analyses, given that only 4 women carried the DD genotype. Table 2 presents the odds ratios (ORs) of maternal lifetime stress on risk of sPTB, stratified by the rs35331017 genotypes. Among women carrying the rs35331017-II genotype, those reporting average and high lifetime stress had 1.5 (95% CI=1.1–1.9, P=0.007) and 2.1 times (95% CI=1.4– 3.1, P=0.0003) increased odds of sPTB, respectively, compared to mothers reporting low lifetime stress. However, in women carrying the rs35331017-ID/DD genotype, those reporting high lifetime stress demonstrated a reduction in the odds of sPTB compared to women reporting low lifetime stress (OR= 0.05, 95% CI= 0.01–0.32; P=0.001). The interacting effects of the rs35331017 genotype and maternal stress are presented in Figure 2, which further demonstrates a dose-response positive association between maternal lifetime stress and risk of sPTB only in mothers carrying the rs35331017-II genotype. We then carried out similar analyses to explore whether there was an interaction effect between the rs35331017 genotype and

maternal stress during pregnancy on risk of sPTB. We found a similar pattern, although the effect size was relatively modest (Table 2 & Fig 2, $P_{G\times E} = 1.2 \times 10^{-5}$).

We further performed sensitivity analyses to assess the robustness of the maternal *rs35331017*× lifetime stress interaction on PTB subtypes. As presented in Supplementary Table 1, the effect size and direction of the *rs35331017*×maternal lifetime stress interaction was comparable for early sPTB (<33 weeks; $P_{G\times E}$ =0.0005) and late sPTB (33–36 weeks; $P_{G\times E}$ =1.3×10^{-6}). Lastly, we stratified our interaction analyses by newborn sex, which revealed that the *rs35331017* × maternal lifetime stress interaction on risk of sPTB tended to be stronger among females ($P_{G\times E}$ =1.8×10^{-6}) than males ($P_{G\times E}$ =0.046) (Supplementary Table 2).

Replication studies and meta analyses

The replication sample includes 337 AA mothers and 738 Caucasian mothers from the GPN study, with their population characteristics shown in Supplementary Table 3. Among the 337 AA mothers, a marginally significant *rs35331017* × maternal stress interaction was observed on sPTB ($P_{G\times E}$ = 0.088), with the association in the same direction as in the BBC. Interestingly, we found that there was a *rs35331017* × maternal stress interaction for sPTB risk in 738 Caucasian mothers ($P_{G\times E}$ = 0.023, Table 3 & Supplementary Fig 2), as well as in the combined cohort of AA and Caucasian mothers (P =0.009) from the GPN study (which included adjustment for maternal ancestry). With the meta analyses combining the discovery and replication samples, we identified an even stronger *rs35331017* × maternal stress interaction on risk of sPTB (P =4.5×10^{-10}).

Discussion

This is the first study to investigate maternal genome-wide gene × maternal perceived stress interactions on the risk of sPTB in African Americans, a high-risk population for PTB. Specifically, we have identified a genome-wide significant interaction between maternal *PTPRD* genetic variants and maternal lifetime stress in AA women from the BBC, and further replicated this interaction in AA and Caucasian women from the GPN study. These findings, if further validated in other large populations, underscore the importance of considering maternal genetic factors and G×E interactions when assessing socio-environmental determinants of sPTB.

Our study may contribute new insight into the relationship between maternal stress and sPTB. To our knowledge, previous studies evaluating associations between maternal perceived stress and risk of sPTB are inconclusive. Some studies have reported maternal perceived stress as a risk factor for sPTB,(8, 11, 14) while others demonstrated a negative association between them.(9, 10, 13) Findings from the current study may help to explain such inconsistent findings. Our data indicated that the associations between maternal lifetime stress and risk of PTB may vary in women with different genetic backgrounds. Among mothers carrying the *rs35331017*-II genotype, those reporting high lifetime stress were at about 2-fold higher risk of sPTB than those reporting low lifetime stress. However, this association was reversed among mothers carrying the DD or ID genotype at *rs35331017*. A similar pattern, but with a relatively modest effect size, was observed for

the interaction between *rs35331017* genotypes and maternal stress during pregnancy on risk of sPTB. Furthermore, we observed that, among all the genotyped mothers, high lifetime stress was more prevalent in mothers of sPTB than in mothers of TB, while maternal stress during pregnancy was only marginally different between these two groups. One possible explanation is that lifetime stress is better able to capture cumulative stress that may affect maternal preconception health as well as health during pregnancy, which is consistent with a life course health framework.

Although the underlying mechanism linking maternal perceived stress with risk of sPTB is not yet clear, several biological pathways have been implicated. First, activation of the hypothalamic-pituitary-adrenal axis, a major endocrine pathway, is activated in response to stress. Maternal stress triggers norepinephrine and cortisol release, which results in activation of placental corticotrophin-releasing hormone (CRH) gene expression. CRH gradually increases as the pregnancy progresses, and serves as a "placental clock" determining the timing of parturition.(31) It has been hypothesized that in mothers experiencing high levels of stress, increased CRH levels may alter the timing of parturition and ultimately promote PTB.(32, 33) Another biological pathway implicated in the pathophysiology of sPTB is inflammation/infection. Higher stress levels are associated with higher circulating levels of inflammatory markers (such as C-reactive protein, interleukin (IL) 1 beta and IL-6), and lower levels of anti-inflammatory cytokines such as IL-10, (34, 35) leading to the T-helper 1 skewing and increased sPTB risk.

The 35331017 × stress interaction was further replicated in Caucasian women from the GPN study, suggesting a shared effect across different ethnic populations. It is largely unknown how the identified maternal *rs35331017* × maternal perceived stress interaction may affect sPTB risk. SNP *rs35331017* is a T-nucleotide insertion/deletion polymorphism located in the intronic region of the gene coding protein-tyrosine phosphatase receptor Type D (*PTPRD*). Protein PTPRD, which is highly expressed in the human brain, plays a potential role in psychopathology because it can bidirectionally induce pre- and post-synaptic differentiation of neurons by mediating interaction with IL1 receptor accessory protein and IL1 receptor accessory protein like 1. (36) Previous studies have linked the *PTPRD* gene with multiple traits, such as substance addiction, (37, 38) and gestational diabetes,(39) which show some degree of association with PTB. For example, It is likely that substance addiction during pregnancy, which may directly or indirectly target placental transport systems between maternal and fetal circulation, may lead to accumulated levels of serotonin and norepinephrine in the intravillous space.(40) Increased levels of these neurotransmitters may be associated with an increased risk of sPTB via altering CRH levels, a shared pathway underlying maternal stress - PTB associations, which may help to explain the interaction effect identified in this study. It would be interesting to further investigate whether the identified *rs35331017* × maternal stress interactions on sPTB risk are mediated by CRH levels.

The implication of G×E interactions in sPTB could be far-reaching. In addition to further elucidating the "missing heritability" of PTB, the discovery of significant G×E interactions creates the opportunity for translational application. For example, our findings, if further confirmed, could prove valuable for the prediction and prevention of sPTB,

since *rs35331017* genotypes and maternal perceived lifetime stress can be measured well before pregnancy and maternal stress is, to an extent, a modifiable factor. Our findings also indicate that effective prevention strategies targeting sPTB may differ for women with different genetic backgrounds. Specifically, women carrying the *rs35331017*-II genotype are at particular risk for stress-related sPTB, and they may therefore benefit from prioritizing stress reduction before and during pregnancy to decrease the risk of sPTB. Conversely, women carrying the *rs35331017 DD or ID* genotype may demonstrate more resilience in the face of acute and chronic stress, and for these women, emphasis on avoiding and mitigating other risk factors may be more effective in preventing PTB. However, these strategies require further investigation.

Several limitations should be acknowledged. First, perceived stress levels during a mother's lifetime and during pregnancy were self-reported by the mothers, which may lead to recall bias. We believe that this bias is largely random and does not significantly affect our G×E findings, since mothers were not aware of their genotypes at the time of report, and the findings were successfully replicated in an independent cohort. Second, other stress-related variables, such as depressive symptoms and adverse life events, are less frequent and thus were not analyzed in the current study due to limited statistical power. Third, the current study may have a limited power to test G×E for SNPs with low minor allele frequencies (i.e., MAF < 2 %) and/or with relatively modest interaction effects with maternal stress. Fourth, the results of the study remain purely associative, with unknown mechanisms, and warrant future investigation. The identified variant, *rs35331017*, is an insertion/deletion polymorphism in an intronic region, whose function is not known. Finally, the BBC was specifically designed to study PTB in a predominantly urban, low-income minority population. Caution is warranted when generalizing our findings to other populations.

In conclusion, this is the first study to demonstrate a significant genome-wide maternal gene × stress interaction on sPTB risk in a high-risk AA population in Boston, MA. Our study highlights the importance of considering genetic factors and G×E interactions when investigating socio-environmental determinants of sPTB. Our findings, if further confirmed, may provide new insight into individual susceptibility to stress-induced sPTB; help advance prediction and prevention of sPTB; and stimulate future studies on the functionality of the identified target gene and SNPs, and validations of this finding in other ethnic groups.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Liu L, et al.Global, regional, and national causes of under-5 mortality in 2000–15: an updated systematic analysis with implications for the Sustainable Development Goals. Lancet. 388, 3027–3035 (2016). [PubMed: 27839855]
2. Lawn JE, Kinney M. Preterm birth: now the leading cause of child death worldwide. Sci Transl Med. 6, 263ed221 (2014).
3. Pritchard MA, et al.Autism in Toddlers Born Very Preterm. Pediatrics. 137, e20151949 (2016).
4. Wang G, et al.Preterm birth and random plasma insulin levels at birth and in early childhood. JAMA : the journal of the American Medical Association. 311, 587–596 (2014). [PubMed: 24519298]
5. Martin JA, Hamilton BE, Osterman MJ, Driscoll AK, Mathews TJBirths: Final Data for 2015. Natl Vital Stat Rep. 66, 1 (2017).
6. Grobman WA, et al.Racial/Ethnic Disparities in Measures of Self-reported Psychosocial States and Traits during Pregnancy. Am J Perinatol. 33, 1426–1432 (2016). [PubMed: 27500932]
7. Wadhwa PD, Entringer S, Buss C, Lu MCThe contribution of maternal stress to preterm birth: issues and considerations. Clin Perinatol. 38, 351–384 (2011). [PubMed: 21890014]
8. Straub H, Adams M, Kim JJ, Silver RKAntenatal depressive symptoms increase the likelihood of preterm birth. Am J Obstet Gynecol. 207, 329 e321–324 (2012). [PubMed: 22789523]
9. Lu MC, Chen B. Racial and ethnic disparities in preterm birth: the role of stressful life events. Am J Obstet Gynecol. 191, 691–699 (2004). [PubMed: 15467527]
10. Kramer MS, et al.Stress pathways to spontaneous preterm birth: the role of stressors, psychological distress, and stress hormones. Am J Epidemiol. 169, 1319–1326 (2009). [PubMed: 19363098]
11. Dole N, et al.Maternal stress and preterm birth. Am J Epidemiol. 157, 14–24 (2003). [PubMed: 12505886]
12. Kitai T, et al.A comparison of maternal and neonatal outcomes of pregnancy with mental disorders: results of an analysis using propensity score-based weighting. Arch Gynecol Obstet. 290, 883–889 (2014). [PubMed: 24927782]
13. Krabbendam L, et al.The impact of maternal stress on pregnancy outcome in a well-educated Caucasian population. Paediatr Perinat Epidemiol. 19, 421–425 (2005). [PubMed: 16269069]
14. Yonkers KA, et al.Pregnant women with posttraumatic stress disorder and risk of preterm birth. JAMA Psychiatry. 71, 897–904 (2014). [PubMed: 24920287]
15. Boyce TW2019The Orchid and the Dandelion: Why Some Children Struggle and How All Can Thrive. (Knopf, New York, 2019)
16. Massey SH, et al.Does MAOA increase susceptibility to prenatal stress in young children? Neurotoxicol Teratol. 61, 82–91 (2017). [PubMed: 28163169]
17. Green CG, et al.Prenatal maternal depression and child serotonin transporter linked polymorphic region (5-HTTLPR) and dopamine receptor D4 (DRD4) genotype predict negative emotionality from 3 to 36 months. Dev Psychopathol. 29, 901–917 (2017). [PubMed: 27427178]
18. Mparmpakas D, et al.Differential expression of placental glucocorticoid receptors and growth arrest-specific transcript 5 in term and preterm pregnancies: evidence for involvement of maternal stress. Obstet Gynecol Int. 2014, 239278 (2014). [PubMed: 24899900]

19. Liu X, et al.Variants in the fetal genome near pro-inflammatory cytokine genes on 2q13 associate with gestational duration. Nat Commun. 10, 3927 (2019). [PubMed: 31477735]
20. Zhang G, et al.Genetic Associations with Gestational Duration and Spontaneous Preterm Birth. N Engl J Med. 377, 1156–1167 (2017). [PubMed: 28877031]
21. Wang X, et al.Maternal cigarette smoking, metabolic gene polymorphism, and infant birth weight. JAMA. 287, 195–202 (2002). [PubMed: 11779261]
22. Hong X, et al.Genome-wide approach identifies a novel gene-maternal pre-pregnancy BMI interaction on preterm birth. Nat Commun. 8, 15608 (2017). [PubMed: 28598419]
23. Wang G, et al.Preterm birth and random plasma insulin levels at birth and in early childhood. JAMA. 311, 587–596 (2014). [PubMed: 24519298]
24. Delaneau O, Marchini J, Zagury JFA linear complexity phasing method for thousands of genomes. Nat Methods. 9, 179–181 (2012).
25. Howie B, Fuchsberger C, Stephens M, Marchini J, Abecasis GRFast and accurate genotype imputation in genome-wide association studies through pre-phasing. Nat Genet. 44, 955–959 (2012). [PubMed: 22820512]
26. Price AL, et al.Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet. 38, 904–909 (2006). [PubMed: 16862161]
27. Purcell S, et al.PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 81, 559–575 (2007). [PubMed: 17701901]
28. Gogarten SM, et al.GWASTools: an R/Bioconductor package for quality control and analysis of genome-wide association studies. Bioinformatics. 28, 3329–3331 (2012). [PubMed: 23052040]
29. Pruim RJ, et al.LocusZoom: regional visualization of genome-wide association scan results. Bioinformatics. 26, 2336–2337 (2010). [PubMed: 20634204]
30. Zhang H, et al.A genome-wide association study of early spontaneous preterm delivery. Genet Epidemiol. 39, 217–226 (2015). [PubMed: 25599974]
31. Vrekoussis T, et al.The role of stress in female reproduction and pregnancy: an update. Ann N Y Acad Sci. 1205, 69–75 (2010). [PubMed: 20840255]
32. Hobel CJ, Dunkel-Schetter C, Roesch SC, Castro LC, Arora CPMaternal plasma corticotropin-releasing hormone associated with stress at 20 weeks' gestation in pregnancies ending in preterm delivery. Am J Obstet Gynecol. 180, S257–263 (1999). [PubMed: 9914629]
33. Hobel CJ, Dunkel-Schetter C, Roesch S. Maternal stress as a signal to the fetus. Prenat Neonat Med. 3, 116–120 (1998).
34. Coussons-Read ME, Okun ML, Nettles CDPsychosocial stress increases inflammatory markers and alters cytokine production across pregnancy. Brain Behav Immun. 21, 343–350 (2007). [PubMed: 17029703]
35. Christian LM, Franco A, Glaser R, Iams JDDepressive symptoms are associated with elevated serum proinflammatory cytokines among pregnant women. Brain Behav Immun. 23, 750–754 (2009). [PubMed: 19258033]
36. Yamagata A, et al.Mechanisms of splicing-dependent trans-synaptic adhesion by PTPdelta-IL1RAPL1/IL-1RAcP for synaptic differentiation. Nat Commun. 6, 6926 (2015). [PubMed: 25908590]
37. Drgon T, et al."Replicated" genome wide association for dependence on illegal substances: genomic regions identified by overlapping clusters of nominally positive SNPs. Am J Med Genet B Neuropsychiatr Genet. 156, 125–138 (2011). [PubMed: 21302341]
38. Uhl GR, et al.Cocaine reward is reduced by decreased expression of receptor-type protein tyrosine phosphatase D (PTPRD) and by a novel PTPRD antagonist. Proc Natl Acad Sci U S A. 115, 11597–11602 (2018). [PubMed: 30348770]
39. Chen T, et al.Genetic variants in PTPRD and risk of gestational diabetes mellitus. Oncotarget. 7, 76101–76107 (2016). [PubMed: 27738328]
40. Baer RJ, et al.Risk of preterm birth among women using drugs during pregnancy with elevated alpha-fetoprotein. J Perinatol. 37, 220–225 (2017). [PubMed: 27929528]

Impact

1. This was the first preterm study to demonstrate a significant genome-wide gene-stress interaction in African Americans, specifically, *PTPRD* gene variants can interact with maternal perceived stress to affect risk of spontaneous preterm birth.
2. The *PTPRD* × maternal stress interaction was demonstrated in African Americans and replicated in both African Americans and Caucasians from the GPN study.
3. Our findings highlight the importance of considering genetic susceptibility in assessing the role of maternal stress on spontaneous preterm birth.

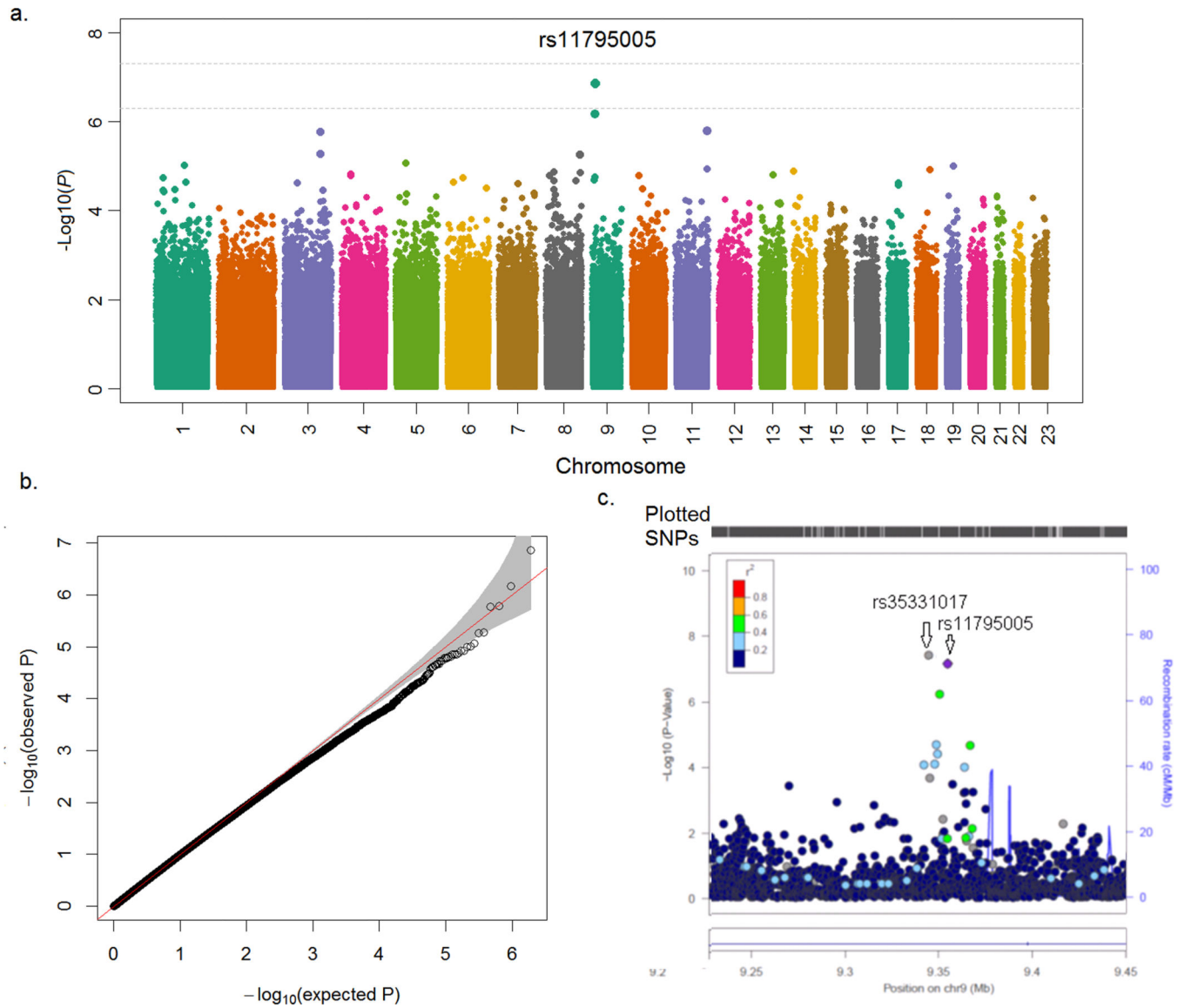

Figure 1. Manhattan, quantile-quantile (Q-Q), and locuszoom plot of the genome-wide interaction associations with maternal lifetime stress on spontaneous PTB, in 1,490 African-American mothers from the Boston Birth Cohort.

a-c manhattan plot, Q-Q plot and locuszoom plot, respectively, for the genome-wide interaction analyses performed using the conventional 1-degree of freedom interaction test based on the multiple logistic regression models, adjusted for genotyping batch, maternal genetic ancestry, age at delivery, parity, marital status, social support from the baby's father and newborn sex.

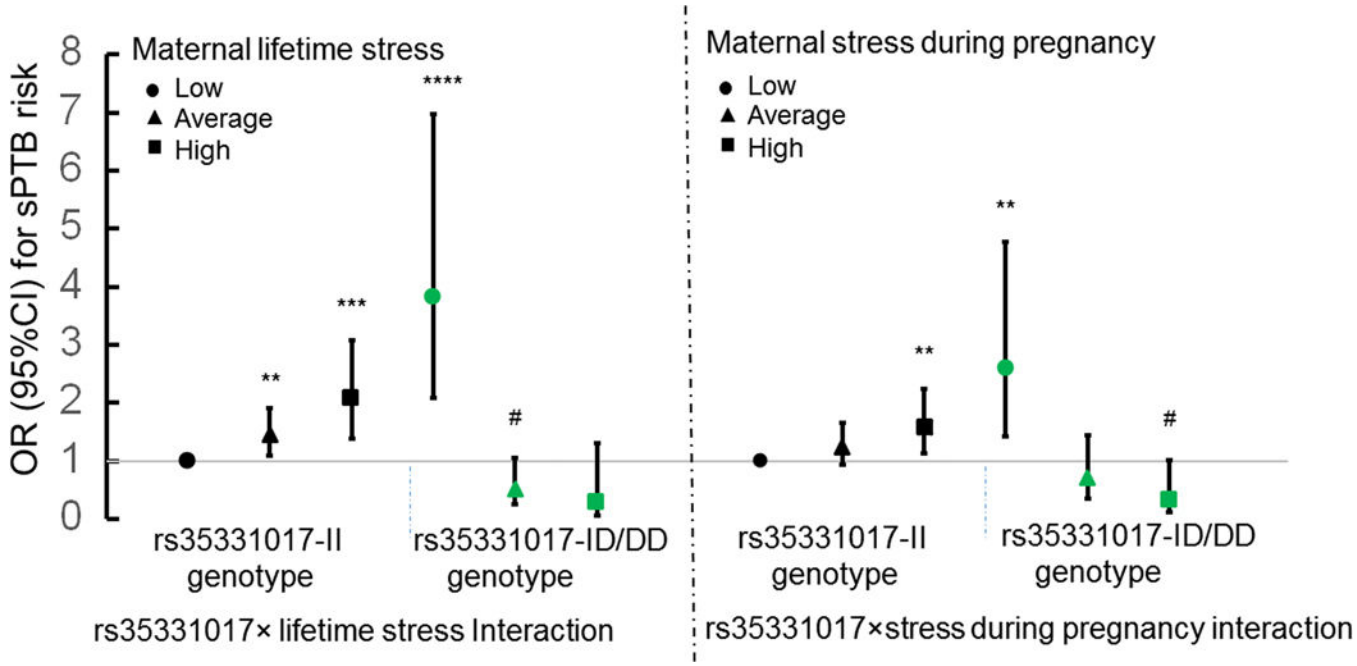

Figure 2. Joint associations between *rs35331017* in the *PTPRD* gene and maternal perceived stress on sPTB in African-American mothers from the BBC.

Y axis reflects the odds ratio (OR) and 95% confidence interval (CI) of sPTB risk for each subgroup stratified by the genotype of *rs35331017* and maternal lifetime stress (Fig. 2A) or stress during pregnancy (Fig. 2B), with low-stress mothers carrying the *rs35331017*-II genotype as the reference group. This analysis was conducted based on multiple logistic regression models adjusted for genotyping batch, maternal genetic ancestry, age at delivery, marital status, parity, social support from the baby's father and newborn sex. # $0.05<P<0.10$; ** $P<0.01$; *** $P<0.001$, **** $P<0.0001$.

Table 1.

Population characteristics of 1,490 African-American mothers from the Boston Birth Cohort

Maternal characteristics [a]		Mothers of TB (Controls)	Mothers of sPTB (Cases)	*P*-value [b]
n		1033	457	
Age at delivery (years), mean ± SD		28.3±0.20	28.5±0.32	0.603
Gestational age at delivery (weeks), mean ± SD		39.6±0.04	33.3±0.17	<0.001
Age at delivery (years), n (%)				0.400
	<20	115 (11.1)	53 (11.6)	
	20–29.9	503 (48.7)	208 (45.5)	
	30–34.9	239 (23.1)	102 (22.3)	
	≥ 35.0	176 (17.1)	94 (20.6)	
Pre-pregnancy BMI (kg/m^2), n (%)				
	<18.5	36 (3.5)	18 (3.9)	0.189
	18.5–24.9	445 (43.1)	192 (42.0)	
	25.0–29.9	261 (25.3)	136 (29.8)	
	≥ 30	233 (22.6)	82 (17.9)	
	Missing	58 (5.6)	29 (6.3)	
Marital status, n (%)	Married	347 (33.6)	124 (27.1)	0.013
Parity, n (%)	0	439 (42.4)	191 (41.8)	0.096
	1	297 (28.8)	112 (24.5)	
	≥ 2	297 (28.8)	154 (33.7)	
Education level, n (%)	College or above	406 (39.3)	160 (35.1)	0.130
Smoking during pregnancy, n (%)				
	Never	853 (82.6)	337 (73.7)	<0.001
	Former smoker	76 (7.4)	41 (9.0)	
	Current smoker	97 (9.4)	78 (17.1)	
	Missing	7 (0.7)	1 (0.2)	
Alcohol drinking during pregnancy		74 (7.2)	43 (9.4)	0.062
Maternal lifetime stress	Low	395 (38.2)	144 (31.5)	0.001
	Average	533 (51.6)	239 (52.3)	
	High	105 (10.2)	74 (16.2)	
Maternal stress during pregnancy				
	Low	384 (37.2)	148 (32.4)	0.071
	Average	459 (44.4)	200 (43.8)	
	High	187 (18.1)	108 (23.6)	
	Missing	3 (0.3)	1 (0.2)	
Support from the baby's father	No or little	177 (17.1)	102 (22.3)	0.001
	Fairy or more	791 (76.6)	310 (67.8)	
	Missing	65 (6.3)	45 (9.8)	
Support from family/friends	No or little	76 (7.3)	40 (8.8)	0.003
	Fairy or more	910 (88.2)	377 (82.4)	

Maternal characteristics [a]		**Mothers of TB (Controls)**	**Mothers of sPTB (Cases)**	***P*-value** [b]
	Missing	47 (4.5)	40 (8.8)	
Newborn sex: Male		526 (50.9)	238 (52.1)	0.680

TB: Term birth; sPTB: Spontaneous preterm birth; BMI: Body mass index; SD: Standard deviation

[a] n (%) are shown in the table, if not specified.

[b] Each variable was compared between sPTB cases and TB controls, using chi-squared and t-test, respectively, for categorical and continuous variables.

Table 2.

Stratified analyses[a] by genotypes of the *PTPRD* rs35331017 variant for the association between lifetime stress and spontaneous PTB in the mothers from the Boston Birth Cohort

Maternal stress	Mothers carrying rs35331017 II genotype				Mothers carrying rs35331017-ID / DD genotype				P_{int} [c]
	sPTB	TB	OR (95% CI)[b]	P[b]	sPTB	TB	OR (95% CI)[b]	P[b]	
Maternal lifetime stress									
Low [a]	116	368	1.0	Ref	28	26	1.0	Ref	
Average	226	473	1.46 (1.11–1.93)	0.007	11	57	0.10 (0.03–0.27)	1.1×10^{-5}	
High	72	91	2.09 (1.40–3.11)	0.0003	2	14	0.05 (0.01–0.32)	0.0014	4.7×10^{-8}
Maternal stress during pregnancy									
Low [a]	123	352	1.0	Ref	23	30	1.0	Ref	
Average	186	415	1.25 (0.94–1.65)	0.123	12	41	0.26 (0.10–0.68)	0.006	
High	104	163	1.60 (1.14–2.26)	0.007	4	23	0.11 (0.03–0.43)	0.0014	1.2×10^{-5}

CI: confidence interval. OR: odds ratio; sPTB: Spontaneous preterm birth; TB: Term birth.

[a]The “low” category as the reference group in each genotype strata.

[b]Adjusted for genotyping batch, maternal genetic ancestry, age at delivery, marital status, parity, social support from the baby’s father and newborn sex.

[c]The interaction effect was analyzed in the total sample by adding lifetime stress, *rs35331017* (under the additive genetic model) and their interaction term into the regression model, with adjustment of the same covariates as mentioned above.

Table 3.

The main effects of maternal *rs35331017*, maternal stress and their interaction effects on spontaneous PTB in the mothers from the Boston Birth Cohort and from the GPN study

Variable	BBC discovery [a] n=1,484[b]			GPN Replication [c] African-American Mothers n=337			GPN Replication [c] Caucasian Mothers n=738		
	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
rs35331017	3.51	1.98–6.22	1.73×10^{-5}	45.6	0.43–482.0	0.108	4.35	1.18–16.5	0.030
Perceived stress	1.44	1.19–1.75	1.75×10^{-4}	1.76	1.14–2.71	0.010	1.78	1.35–2.56	0.0005
rs35331017×stress interaction [d]	0.14	0.07–0.28	4.7×10^{-8}	0.12	0.01–1.37	0.088	0.46	0.20–0.86	0.023

BBC: Boston Birth Cohort; CI: confidence interval; GPN: Genomic and Proteomic Network for Preterm Birth Research; PTB: preterm birth; OR: odds ratio.

[a] The analysis was conducted using the logistic regression model, adjusted for genotyping batch, maternal genetic ancestry, age at delivery, marital status, parity, social support from the baby's father and newborn sex in the BBC discovery sample.

[b] n=1484 after removing 6 mothers with missing genotypic data on rs35331017.

[c] The analysis was conducted using the logistic regression model, adjusted for genetic ancestry, maternal age at enrollment, parity, marital status and newborn sex in the GPN replication sample.

[d] Here *P* value for the interaction effect was estimated using an interaction term of maternal stress and the 35331017 genotype (under an additive genetic model).